IN THE UNITED STATES DISTRICT COURT FOR THE

NORTHERN DISTRICT OF OKLAHOMA

W. A. DREW EDMONDSON, in his capacity as ATTORNEY GENERAL OF THE STATE OF OKLAHOMA and OKLAHOMA SECRETARY OF THE ENVIRONMENT C. MILES TOLBERT, in his capacity as the TRUSTEE FOR NATURAL RESOURCES FOR THE STATE OF OKLAHOMA,	)	
Plaintiff,	)	
vs.	)	4:05-CV-00329-TCK-SAJ
TYSON FOODS, INC., et al,	)	
Defendants.	)	

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THE DEPOSITION OF TAMZEN WOOD MACBETH, produced as a witness on behalf of the Defendants in the above styled and numbered cause, taken on the 30th day of October, 2008, in the City of Tulsa, County of Tulsa, State of Oklahoma, before me, Marlene Percefull, a Certified Shorthand Reporter, duly certified under and by virtue of the laws of the State of Oklahoma.

staffed in-house? 9:02AM

A Yes.

Q Is that right? Okay.

A Now, for clarification -- so, for instance, the DNA sequencing component of it, we do submit those to 9:02AM the molecular research core facility and they actually do the DNA sequencing. So I guess when you say were there other labs that did a component of the work for that particular component, they also run the T-RFLP analysis for us because that has to be run on a DNA 9:03AM sequencer as well.

Q Was there any other part of the project that occurs to you that was done by anyone outside of North Wind?

A Not that I can think of specifically. 9:03AM

Q When did North Wind first get involved in this case?

A We first got involved in the spring of 2005, or that's the first that I heard of it, I believe.

Q Okay. How did North Wind's involvement come 9:03AM about?

A Kind of an interesting progression. During my graduate work at the Idaho National lab, when I was developing a lot of molecular techniques that we were using to characterize these hazard waste site microbial 9:04AM

she, through collaboration with Roger Olsen, constructed the sampling strategy that I describe before where she said, okay, I need to be ensured by testing this many cattle scats and this many goose scats and duck scats and swine scats and human scats that -- and we -- that I can be assured that within this basin we don't see the marker. 9:56AM 9:56AM

Q So do you recall discussing with her specifically then the need to have, you know, a representative number of each of these? 9:56AM

A Yes.

Q Okay. And was she of the view to extend -- do you recall, was she of the view that the various scat samples from other animals that were tested, each of those was of a sufficiently large number to -- to accurately categorize the basin? 9:57AM

A That I am not -- we didn't discuss specifically.

Q Okay. Do you remember yourself giving thought to whether you had enough cattle or goose or duck samples to accurately categorize those populations? 9:57AM

A No.

Q You also used the term "utility" as -- when you were giving me the list of things that Dr. Harwood helped you with. What do you mean by "utility"?

A Well, in this case, the marker had to be useful in 9:57AM

A No. 10:31AM

Q So -- and the reason I ask that is because you may or may now know that Dr. Olsen in this case is sponsoring a principal component analysis, which includes a number of different components, metals and 10:31AM chemicals and such?

A Right.

Q Am I correct that you had no involvement in that?

A That's correct.

Q What does the term fate and transport mean to you 10:31AM in the microbiology context?

A It generally means, in our world, you know, what -- whatever your particular microbe of interest is, where that microbe is growing, what that microbe does in the environment, how that microbe is 10:32AM transported in the environment, generally.

Q Does it also include what factors lead to its death?

A Sure.

Q Okay. That would be the fate part? 10:32AM

A Uh-huh.

Q Okay. Did you conduct any fate -- any study of the fate and transport characteristics of any bacteria for this project?

A No. 10:32AM

data in this context, so I should be clear about that. 10:33AM

Q Uh-huh.

A We use these data on our other projects and for our other targets to look at fate and transport with these DNA techniques, but has not -- what we do has not 10:34AM been applied to this project.

Q I think I lost you in the middle there somewhere. When you talk about other projects, do you mean projects other than the chicken farm project?

A Uh-huh. 10:34AM

Q Within the context of this project, are you aware of anyone studying how the organism that carries the biomarker sequence, how it moves in the environment?

A Jody and Roger are evaluating where we are seeing the presence of the marker in the environment. 10:34AM

Q And tell me how they're doing that.

A The only thing that I've seen is some spatial maps and some correlations with E. coli and enterococcus. I'm not involved in the particular analysis that they're doing. 10:35AM

Q Okay. So if you take a sample of point A and you find the biomarker, and you take a sample of point B and you find the biomarker, are you aware of anyone conducting any study to tell how -- you know, say the Brevibacteria that carries the biomarker got from point 10:35AM

A to point B or if it got from point A to point B? 10:35AM
A I'm not aware of any.
Q Okay. Thank you.
MR. TODD: Let me have you mark this as Exhibit 1. Where are we on time? 10:35AM
MR. BULLOCK: Did you get an answer to your question?
THE VIDEOGRAPHER: Ten minutes.
Q Dr. Macbeth, I've handed you what's been marked as Exhibit 1. Do you recognize this document? 10:36AM
A Yes.
Q What is this document?
A This is the detailed report that we provided on the -- on an overview of the development of the biomarker. 10:36AM
Q Okay. This report is dated December 2007. There were various drafts in the materials that were provided.
A Yes.
Q But this seemed to be the latest one. Do you know 10:36AM for certain whether the December 2007 version of this report is the final version?
A I believe it is, yes.
Q Are you familiar with this report?
A Yes. 10:37AM

would you expect them to have similar or different microbial populations? 10:57AM

A Well, what do you mean on the same farm?

Q Well, let's break it down even further. Let's say the same chicken house. Let's say they were taken from right next to each other, scoop A and scoop B, would you say they have similar or different microbial populations? 10:58AM

A If the characteristics of the litter are the same and they sampled it in exactly the same way, I would anticipate that some of the populations would be similar, yes. 10:58AM

Q Would you -- let's say you took a sampling from a chicken house in Arkansas and a sample of litter from a chicken house in Delaware, so I don't know how far, maybe 1,000 miles apart, would you expect to get -- generally speaking, in your experience as an expert in this area, would you expect -- 10:58AM

MR. BULLOCK: Object to form.

A Yeah. I just want to clarify, I'm not an expert -- 10:58AM

Q Okay.

A -- in microbiology of feces, so I don't think I can answer questions regarding the microbiology of feces. 10:59AM

sampling? 11:00AM

A Yes.

Q Do you know, we talked earlier about with regard to the animal scats, that one concern of Professor Harwood's was to try to get a number that would 11:00AM characterize the entire watershed. Do you have any recollection of any similar discussion regarding litter samples?

A No.

Q I have handed you what has been marked as Exhibit 11:01AM 2. Are you familiar with this document?

A It an e-mail. So "by familiar," do you mean does it look like something I would have received? Yes.

Q I'm correct that you don't remember receiving this particular e-mail on January 31st, 2006? 11:01AM

A Yes.

Q Okay. All right. I'll represent to you that this came from the e-mails that were produced to us.

A Okay.

Q And obviously your lawyers can check that later, 11:01AM so if you'll accept that with me --

A Sure.

Q -- we will move along much faster. This is an e-mail from you to Roger Olsen, is that correct? I'm sorry, to you from Roger Olsen. 11:02AM

A To me from Roger, yes. 11:02AM
Q Copies Kent Sorenson. And in the first line there of the text, I'll read it, it says, "We will be sampling litter and oil at one to two farms this Thursday and Friday." Did I read that correctly? 11:02AM
A Yes.
Q Do you have any reason to question that litter samples were taken at any place other than one to two farms?
A No. 11:02AM
Q Exhibit 3 is again an e-mail --
A Uh-huh.
Q -- that was produced to us as part of materials that came from North Wind. Do you have any recollection of this e-mail? 11:03AM
A I do not.
Q For the record, I should just say that the name on the top there, Ann Elizabeth Gedicks, is my paralegal and I'm not sure why in the printing process her name appeared, but that is not, obviously, not part of the 11:03AM document that was produced.
If you look down at the text of this e-mail, do you see FAC-01B? It's the second sample listed.
A Yes. 11:03AM

Q And the text reads, "This is a co-located sample of FAC-01A"? 11:03AM

A Yes.

Q So this e-mail was again from Roger Olsen to you, as well as Sorenson and Dr. Harwood, copied to David Page. What do you take co-located to mean? 11:03AM

A I don't know.

Q When you received this e-mail you had no idea what that meant?

A I would presume that it was a sample that was located somewhere close to FAC-01A. 11:04AM

Q I find the sample name as confusing as you do.

Do you believe that litter samples taken -- let me back up. Assuming that sample FAC-01A and FAC-01B were taken from the same place or closely located places, do you believe that those litter samples accurately characterize litter throughout the entire Illinois River Watershed? 11:04AM

A I can't speak to that.

Q What would you have to know to speak to that? 11:05AM

MR. PAGE: Object to the form.

A Like I said, my expertise is not in fecal bacteria, so I can't really even speculate a guess at this point.

Q Okay. The purpose of your mission here was to 11:05AM

develop a poultry litter specific assay, right? 11:05AM
A Yes.
Q And Exhibit 1 is your writeup how you went about doing that?
A Uh-huh. 11:05AM
Q Right. And on the very first page you start by noting where the DNA that you're extracting from poultry litter samples, where that came from?
A Yes.
Q If you're going to attempt to develop an assay 11:06AM starting with that litter that can be used to track something and poultry litter anywhere in this watershed, don't you think it's important or do you think it's important to start with a representative litter selection? 11:06AM
A I do. It's just that it was not our purpose to make a decision about whether we thought it was representative or not.
Q Okay. And I understand that that was not your responsibility and I -- and I'm not suggesting that I 11:06AM do think that was your responsibility. I'm just trying to understand the process that you went through in developing this assay. Okay. The next step in this process -- or a next step, I should say, involved something called BLAST? 11:06AM

send us a bill for those copies, we'll cover those as well. 11:11AM

MR. BULLOCK: I bet we'll just put it on the pile.

MR. TODD: Chances are. 11:11AM

Q While we're waiting for that, we'll get that remarked and we can go back to that in a bit, but let me ask you some questions which I don't think you need to look at the exhibit to answer.

What is BLAST? 11:12AM

A BLAST is a database that was developed by the National Center for Bio-informatics something that is essentially a database of all known and unknown microbe -- or, DNA sequences really. It contains a lot of things. We use it a lot during our DNA sequencing and processing to evaluate unknown sequences against this database because it does contain, as I said, all known sequences. 11:12AM

Q What are the -- are the criteria for getting -- personally being included in BLAST? 11:12AM

A Well, in general, when I've submitted DNA sequences to BLAST it's generally in format, you know, when you're ready for a publication. And a lot of times anymore to get a paper published you have to submit your sequences to BLAST and get numbers for 11:13AM

them. But in terms of is there a QAQC about the quality of the sequences that go in, no. 11:13AM

Q Are there other databases that provide, you know, a similar service?

A The Ribosomal Database Project is another database that we use quite a bit that provides similar types of information. 11:13AM

Q You didn't use that in this case, you just used BLAST, is that right?

A We did use the Ribosomal Database Project as well. We generally do both. 11:13AM

Q What do you use that for?

A The RDP has -- so during the primer design phase, it has a program where you can take the primer that you're proposing to develop and search and CBI BLAST does this, too, search what organisms that would target. And, you know, what pieces of DNA essentially that would amplify within the organisms within those databases. So we used RDP for that function, as well as to evaluate various restriction enzymes during the T-RFLP process to see how those enzymes would generate different T-RFLP fragments. 11:13AM 11:14AM

Q Now, you can have a DNA sequence without knowing the organism it comes from?

A Yes. 11:14AM

Q But you indicated that in BLAST that sequences are tied to organisms, is that right? 11:14AM

A Yes.

Q So anything included in BLAST -- any sequence in BLAST, do they all come from a known organism? 11:14AM

A No.

Q They don't? Okay.

A No.

Q How would I --

A Known in terms of culture. Is that what you mean, just for clarification? What do you mean by known versus unknown organisms? 11:15AM

Q Let me -- to me -- well, let's take that culture. Does something have to have been cultured to be in BLAST? 11:15AM

A No.

Q How would a sequence be identified and included in BLAST then when it wasn't cultured?

A So what we generally do is implement or we have collaborators, I should say, that implement processes called phlogogen, phlogogenic analysis. That's where you take known culture organisms, as well as unknown organisms within those databases, and you input those sequences into software. You align your unknown sequences with those known sequences and the software 11:15AM

generates -- has an algorithm in it that generates relationships or similarities between those sequences and so it infers a relationship between your unknown sequences and the known sequences. 11:15AM

Q Okay. And so then which is going to be listed in BLAST as being 98.5 percent related to X cultured organism? 11:16AM

A Yes.

Q Okay. Who submits things to BLAST?

A Generally researchers, primarily. 11:16AM

Q Okay. So I would say if someone was doing a project not dissimilar to your project here and they identified a sequence and determined that it was, you know, closely rated to some known culture organism where they could submit that to BLAST with that information and it would just be included? 11:16AM

A Yes.

Q Okay. And does BLAST tell you -- does it give you the origin of the sequences that it's spitting back at you? 11:16AM

A It does.

Q So who submitted it?

A Yes, if it's published or unpublished, any description that the person included, including the project type, the types of samples, so you can look for 11:17AM

all that information. 11:17AM
Q Okay. Have you had -- well, how much of the bacteria world do you think has been sequenced?
A I think that's really a hard question to answer. I don't think very much, but I can't really ascertain a 11:17AM guess in terms of, you know, how much or how little has been, but --
Q Okay. But would it be fair to say that there's a whole heck of a lot, you know, out there in the world of bacteria that hasn't been sequenced and is not in 11:17AM BLAST?
A Yes.
MR. TODD: Okay. For the record, we now have a proper copy of what I had marked as Exhibit 1. Do you guys mind if we just sub this in? 11:18AM
MR. BULLOCK: Why don't we do this as 1A?
MR. TODD: Okay. That's a good idea.
MR. PAGE: Since you already asked a question on the first Exhibit 1, that is probably wise to do. 11:18AM
MR. TODD: Very good. And just to make the record clear, I have no intention in asking any questions at all about Exhibit 1. All my questions will be about Exhibit 1A. So from here on out, if I say Exhibit 1, I mean 1A. 11:18AM

that as I look at the LA35 list, these are the top ten closest related cultured bacteria that BLAST identified in terms of how closely related what is in BLAST to the LA35 sequence? 11:25AM

A Yes. 11:25AM

Q And so this list, this result would be limited by the limitations inherent in the BLAST database?

A Limitations being?

Q Well, to the extent that something is not in the BLAST database, it's not going to be in the BLAST report, correct? So to the extent that another organism is out there that's not been sequenced carries closely-related or even identical sequence to the biomarker to the LA35 sequence, it wouldn't be in this report, correct? 11:25AM 11:26AM

A Yes, if it -- yes.

Q If it hasn't been --

A Cultured.

Q -- sequenced and submitted to BLAST?

A Yes, correct. 11:26AM

Q Okay. I think I asked you this earlier, but tell me again. You never cultured the organism that carries the biomarker?

A That's correct.

Q Is that something you could have done? 11:26AM

Brevibacterium? 11:35AM

A You can take the primers that you design, so you identify regions of variability within the DNA. Then the regions that you target depend, like I said, on a specificity that you want. You then can take those 11:35AM primers and you can actually run the primers in BLAST and it will compare those primers to all the sequences in the database and say with this primer this is what you would amplify or not amplify. You then refine the design based on those results and -- and then test the 11:35AM primers.

Q And look at Table 4 here on Page 8, if you look at the finding for clone 35, LA35, it says "primer sequence did not match any organisms in the database."

A Yes. 11:35AM

Q So am I correct what you've done there is you created a primer sequence that will reproduce only clone LA35 and not anything that's in the BLAST database?

A Yes, so it -- when it looks for it, it's strictly, 11:36AM you know, are there 100 percent matches to your primer so that's correct.

Q And to the extent that something is not in the BLAST database.

A Correct. 11:36AM

Q Let me ask the question. To the extent that something is not in the BLAST database, you couldn't say whether or not the primers will reproduce it, is that right? 11:36AM

A Correct. So if it's not in the BLAST database, we cannot say whether or not the primers would reproduce it at this stage. 11:36AM

Q Right. Go to Page 11. And the caption here on Section 3.2 is, "Test PCR primers at LA35 against a closely-related bacterium." Tell me what's going on here. 11:37AM

A Let me just make sure I'm with you. Did you say Page 10?

Q Eleven. That's the caption I just read.

A Okay. Okay. So what we did is we looked at the most closely-related sequences, we designed the primers such that they were very specific. And then this is the stage where you test the primers to ensure that they are amplifying one to target, so the LA35 in the case. You can also test it on closely-related or bacterium that you've identified. And in this case we tested it on Brevibacterium species CHNDP 32. Now, this was a sequence that was submitted to BLAST and I think it was the fourth closest match overall of all sequences, both environmental and cultured organisms in 11:37AM 11:37AM 11:38AM

A The --
MR. BULLOCK: Object to form.
A She is the expert in the field and so we discussed it, but she was the -- she was the lead.
Q Okay.
A So she was the one that made the decision. She's the one that decided what to sample, when to sample, how many samples.
Q You do recall discussing this with her, though?
A Yes.
Q Do you recall what -- what criteria, if any, went into deciding which animals to test and which animals not to test?
A I was not privy to those particular conversations.
Q Okay. So you were just given -- the samples showed up at North Wind, is that basically it?
A Yeah, we had a call and she said -- I was on the call when they were discussing, you know, samples to collect and what they had -- decisions, some of the decisions they had come to. Like I said, I wasn't involved in the entire process. And then we were informed on what samples we would be receiving and, you know, how to process them.
Q Do you recall having any input into the decision as to which samples to collect?

the watershed among the 31 fecal samples tested. The Brevibacterium species clone, LA35 PCR primers, did not amplify product in the beef or dairy cattle, swine or human fecal samples. The other three potential biomarkers exhibited amplification to varying degrees in all the fecal samples tested. Given the abundance of Brevibacterium species, clone LA35, in litter and soil samples, and its lack of presence in other fecal samples, this biomarker has been shown to be specific to poultry litter." 12:50PM

Q Do you agree with the last statement there that this biomarker has been shown to be specific to poultry litter?

A Yes.

Q Do you think that is a 100 percent accurate statement? 12:50PM

A I think that it's an accurate statement within the context of the samples that we analyzed.

Q Explain to me what you mean by that.

A We have -- we had a series of samples that we were using to identify the Brevibacterium and to evaluate its specificity. So within that sample set, we feel confident that the assay was specific. 12:51PM

Q Now, when you say "specific," did that mean unique? 12:51PM

you're saying? 12:54PM
A Yes.
Q And would that context -- would that context include the fact that we discussed earlier in designing the primers, you were limited to sequences that were 12:54PM identified in the BLAST database?
A Yes.
Q Would that context include the limitation that results from the fact that only a small number of other types of animals were tested? 12:55PM
A Could you restate that?
Q Sure. When you say it's specific to poultry litter, you mean as compared to geese, ducks, cows, humans and pigs?
A Yes. 12:55PM
Q Okay. Let's move on to Section 4.1 of this report. Section 4, which I believe starts on Page 17. And this is where you start developing the qPCR primer, the quantitive aspect of the assay, is that correct?
A So the primer is the same, but we are developing 12:55PM the quantitative part of the assay, yes.
Q How does a -- you may have just answered this. Does a qPCR primer differ at all from a regular PCR primer?
A No. 12:56PM

A What did it say, it was 98 percent similar so you figure, what is it, about a 530 base pair product. Probably roughly ten or 12 base difference. 1:10PM

Q Okay. Do you know what the closest difference was that you managed to measure? Did you use a melt curve? 1:10PM

MR. BULLOCK: Object to form.

Q Did you use a melt curve to distinguish your sequence from anything other than KCI?

A We used the melt curve to identify whether or not we see other things being amplified in the environmental samples. 1:10PM

Q Okay. And you had -- were there some samples where you did notice -- where you did see a second melt curve?

A I believe there was one sample. 1:10PM

Q Okay. When that happened, did you sequence the -- whatever was producing the other melt curve?

A We didn't at that point, but it is part of our reporting procedure that we always report that there was something else that was amplified. 1:11PM

Q Okay. I have handed you Exhibit 5, which is several additional pages from the lab notebooks that were produced to us. Go ahead and take a second to flip through, then I'll ask you some questions.

A Okay. 1:12PM

Q Let me get you to flip to the second page of this packet, which is numbered as Page 120 on the top left. In the chart here under Task 2 at the top of the page, in the description column on the right-hand side, for several of these -- well, let me ask you this first. Can you tell us what we're looking at here? What is this chart? 1:12PM 1:12PM

A The table or the chart?

Q The table under Task 2.

A So it is some samples, the cleanup method, whether the qPCR reaction amplified the samples, whether the nested qPCR reaction amplified those samples, and then some notes about whether the biomarker was present. 1:13PM

Q Okay. In that notes column, if you'd look at the fourth entry down, which is sample RS-75-050207 A. If you look at the description, it says, "Uncertain if biomarker is present due to melt peak shift -- we could potentially determine biomarker presence with nested qPCR." What is melt peak shift? 1:13PM

A Without knowing what was before or after this, I'm not -- or before it, at least, I'm not exactly sure. I presume that it means that there was a difference in the melt peak relative to what was expected. 1:13PM

Q Okay. And then it says, "We could potentially determine biomarker presence with nested qPCR." Do 1:14PM

you -- why would nested qPCR help you in this instance? 1:14PM
A Without looking at the actual data, I'm not exactly sure, so let me see. I believe -- so it looks like it's referencing this melt curve. So you have RS75, which I believe is the first series of melt 1:14PM curves. So, let's see, in that instance, this is a representation of something that you might see in a sampling if you're amplifying things that are other than perhaps your marker. So given that you have a lot of different peaks, they're sort of shifted all over 1:15PM the place, we could not tell in this sample whether or not the biomarker was present. And in terms of doing the nested qPCR approach, it may just be that the thought was if we could amplify it to greater extent perhaps this was a low yield reaction, for instance, or 1:15PM right at our detection limit for the qPCR method. If that's the case, then sometimes running the nested PCR or nested qPCR will allow you higher concentration essentially, so it would distinguish that.
Q From your initial reaction, it seems is the 1:15PM term -- is the term melt peak shift not a term that has a specific meaning for you?
A It means the melt peak was shifted but relative to some value that you expected.
Q That's what it always means, always relative to an 1:16PM

expected value? 1:16PM
A Yes. I would presume so, although, like I said, I didn't write the note, so --
Q Okay. But you did -- you did tell me at the outset that you went through Dr. Weidhass' lab book with her carefully at the time you were developing this? 1:16PM
A Yes.
Q So I'm assuming that you have some level of familiarity with this? 1:16PM
A Yes.
Q But if you don't, let me know.
A No, no, I do.
Q Flip to the next page, Page 121. It says at the top, can you read the title there? 1:16PM
A "Summary of Talk with Bio-Rad Regarding Reproducibility and Variability in Melt Peaks with Chromo-4."
Q What is Bio-Rad?
A Bio-Rad is the vendor that we get our instrumentation from. 1:16PM
Q What instrumentation?
A The MJ Chromo-4 quantitive PCR machine.
Q And it seems that the question, if I'm reading this correctly, it seems the question as posed to 1:17PM

Bio-Rad, why do CDNA melt peaks shift between nested qPCR and PCR? 1:17PM

A Yes.

Q Am I reading this correctly that the bullet points there are the answers that you got from Bio-Rad? 1:17PM

A Yes.

Q The first one, there's an up arrow. I assume that's greater than, is that fair?

A Or high --

Q High? 1:17PM

A -- concentration.

Q Why don't I have you go ahead and read the first bullet point so I'm not characterizing it.

A So "Why do CDNA melt peaks shift between nested qPCR and PCR? Because there's a higher purity of PCR products versus genomic DNA." 1:17PM

Q Can you explain to me what that means?

A Again, I didn't -- I didn't write it, but I would take that to mean that PCR products are amplifications of your original environmental sample. So especially once you do the purification steps, those PCR products are going to be a higher purity in terms of containing, you know, just DNA versus other things. 1:18PM

Q Okay. The second bullet point says, "Salt concentration differences." Do you see that? 1:18PM

A Yes. 1:18PM
Q Why would that result in a melt peak shift?
A Why would it?
Q Uh-huh.
A A salt concentration in molecular biology in the 1:18PM context of DNA in particular stabilizes DNA or can stabilize DNA. So variability in some salt content or in salt content can increase the stability of your double stranded DNA, for instance. So it may -- as a result, that may affect when that DNA actually melts. 1:19PM
Q So if the same sample was run twice and the only difference between them was the difference in salt concentration, that could lead to a different melt peak?
A It could be shifted, yes. 1:19PM
Q Read the third bullet point for me, if you would.
A "Other DNA and RNA and nucleotides (residual from the PCR) can affect amplification, but that would shift the -- the Ct values, not the melt peak temperature."
Q Explain to me what this means. 1:20PM
A I think she was essentially just writing the list of things that Bio-Rad suggested could impact melt temperatures. And one of the things that they said is that other DNA and RNA or, you know, or sequences, other -- for instance, in PCR reactions, you have the 1:20PM

primer sequence itself, variability in concentrations in that, they suggested, could shift the melt temperature. However, as she notes here, if that's the case, we should also see a shift in the Ct values or where it's coming off in standard curve and not the melt peak. 1:20PM 1:20PM

Q Okay. Then the fourth bullet point says, "DNA binding protein? Longer to linearize." Did I read that correctly?

A Yes, linearize. 1:21PM

Q Can you translate that for me?

A So again, protein and things like bovine serum albumin, which is high protein content, are often added to PCR reactions to stabilize double stranded DNA, so protein content can also impact stability of DNA. 1:21PM

Q Okay. And so these are all things that could be variables that could result in the same sequence resulting in different melt curves?

A Yes.

Q Okay. The next line there appears to be another question, which I'm assuming from the context, you posed to Bio-Rad. Tell me if you disagree with that. It says, "How much variability acceptable within one run of CDNA." Do you see that question? 1:21PM

MR. BULLOCK: Objection to form. 1:22PM

Q Did I read that correctly? 1:22PM
A The -- yes.
Q Okay. And then the response, the bullet point underneath read, "Up to 0.5 degrees C"? What do you take the question to be asking about when it talks 1:22PM about variability acceptable within one run?
A So this is what we were discussing earlier, which is within a replicate set how much variability with -- or shift in that melt temperature would you see and the answer is .5 degrees C. 1:22PM
Q Is it how much you would see or how much was acceptable?
A So within our criteria where we say is this our marker or not, we accept .5 degrees C variability.
Q Okay. Because -- is that because there will 1:22PM likely be some variability but this is just a threshold to where it should cause you concern, is that right?
A Yes.
Q Okay. Skip over the next question then let's go down to the one after that, where it says -- as I read 1:23PM it, tell me if I read incorrectly. Where it says, "Can you quantify a shoulder of a melt peak." Do you see that?
A Yes.
Q What is that question asking? 1:23PM

A This was, I believe, prior to optimizing our SYBR Protocol. So actually if you refer back to Page 21 of the report on Figure 11 where it says the effective DMSO on SYBR Green. When we were initially running the protocol without DMSO, we saw a shoulder in our standards, which is shown on that Figure 11. And so, you know, one of the questions was, well, is that okay? Can we deal with that? Can you quantify it if that's the case or if you are getting, you know, multiple peaks, can you distinguish between being able to quantify those? So that was the question at the time. We optimized the qPCR to eliminate the shoulder and the way that we operate now is that we just report that there is and we do not try to quantity it. 1:23PM 1:23PM 1:24PM

Q When you say you optimize the qPCR to eliminate that shoulder, what did you change in the process to effect that? 1:24PM

A In this instance, we added DMSO.

Q And explain to us what that is.

A DMSO is a compound that, again, helps stabilize double stranded DNA. So it, in this case, made it so that you were getting more uniform denaturing of that DNA sequence. 1:24PM

Q Uh-huh. And does the -- what's the measurement for DMSO? How much that is added? 1:25PM

A I'd have to go back and look and see exactly. I know we ran a series of experiments with varying concentrations of DMSO to determine an optimal concentration. 1:25PM

Q I didn't mean exactly how much to use. I meant, what is the measurement of concentration? I just want to get the terminology right. What is the measurement of concentration for DMSO? What -- 1:25PM

A Like micromolar.

Q That's the unit that it's measured in? 1:25PM

A When we put it on our PCR, we do target a micromolar concentration.

Q Okay. So if I said how many micromolars of DMSO would you add, would that question make sense to you?

A Well, it's not micromolars, it's what -- what is the concentration of DMSO in micromole. 1:26PM

Q Okay. Read on down to the next -- back on Exhibit 5, Page 129. This is the lab report, the page we were looking at before. Would you read the next question after the "can you quantify a shoulder" question, can you read the next one there? 1:26PM

A "Most likely the melt peak shifts between qPCR and nesting qPCRs seen in the report dated 6-14-07 are due to differences in concentration of DMSO added."

Q How does a change in the concentration of DMSO 1:26PM

affect the melt curve? 1:27PM
A It depends.
Q On what?
A I'm not exactly sure how to answer the question.
In our previous discussion, I showed an example of it 1:27PM
not having DMSO. So we have this issue with the
shoulder versus having DMSO where it stabilized the DNA
and we were able to get a consistent melt peak. So
that is an example of an instance that -- of what the
impact of DMSO is. 1:27PM
Q Okay. Think about this the way that I, as a
nonscientist, would look at this. You have the first
curve that you showed us in the report where there's
the little shoulder and you add this stuff and then you
run the processes again. And then magically, from my 1:27PM
perspective, you've got a straight line in the
shoulder. And what you're telling me is that the
addition of this DMSO stuff is what made that straight
line instead of the line with the shoulder. Why?
MR. BULLOCK: Objection to form. 1:28PM
A The -- again, DMSO stabilizes DNA, so --
Q What do you mean by stabilizes DNA? I'm trying to
understand that.
A I would have to go back and look at the exact
mechanism that it's stabilizing the DNA, but in our 1:28PM

case, we're amplifying a relatively long stand of DNA. 1:28PM
Q Uh-huh.
A 500 base pairs is relatively long. So the -- the addition of something that helps you to maintain that double stranded DNA, that length of double stranded DNA 1:28PM helps it to stay together, basically. I'm not exactly sure how else to answer it. I'm not sure what.
Q I think you just have. You mean literally that this strand remains a consistent hole instead of breaking up into little pieces. Is that what you mean? 1:29PM
A Or twisting up, yes, the structure.
Q Okay. That I can conceptualize a little bit. Thank you.
MR. PAGE: Morphology.
Q In the answer to the -- or the explanation below 1:29PM what you just read, as I read this, it says "Problem in that DMSO variability will affect the standard Ct values." Do you see that?
A Yes.
Q Can you read the next sentence? 1:29PM
A "That is if you have a lower DMSO concentration. Ct will be later and you will say there is less DNA present."
Q Explain to me what that means.
A I'm not exactly sure. Given that I didn't write 1:30PM

this, I would only be speculating, I guess, if I responded. It's my speculation that if you have -- so essentially everything that you add to a PCR reaction potentially impacts how efficient that reaction is, so presumably -- and this statement is that variability in DMSO concentrations will ultimately impact how that PCR reaction proceeds. So higher concentrations could increase or decrease the amplification process simply. So in this, it's really just exploring what the impact is of DMSO could mean in terms of the overall amplification process. 1:30PM 1:30PM 1:31PM

Q How do you know how much DMSO to add?

A DMSO is a very widely used compound within molecular biology to stabilize DNA. And in particular, large stranded DNA like we have here. So it is a standard within the industry. You have a suite of compounds that you can use to optimize your PCR and that's one of them. 1:31PM

Q Okay. But how much -- how do you know what concentration to add? 1:31PM

A We review published literature.

Q Okay. Is the -- was the amount or -- sorry. Was the concentration of DMSO that you used uniform across all the melt curves you were in?

A Once we had a standard protocol -- and really all 1:32PM

of this is just us trying to develop the final formulation that we were going to go forward with. 1:32PM

Q Fair enough.

A The -- when we record final results, all of those analyses were conducted in exactly the same way with exactly the same concentrations of all compounds involved, including DMSO. 1:32PM

Q Okay, good. Exhibit 6, which you've been handed, Dr. Macbeth, is a booklet of e-mails which were produced to us from North Wind, which -- all of which come from your e-mails or from Jennifer Weidhass' e-mails. And these all relate to the development of the melt curve process. And I just want to go through a few items here. In the very first one, which is an e-mail from June 6, 2007, from Jennifer Weidhass to you, she says at the top, "Here's another thing we should discuss, our cell recovery is greater than 100 percent," then there's a chart. Can you explain to me what the issue is here? 1:33PM 1:33PM

A This is a chart that was generated during the -- let's see, I have to go back to the original biomarker development report because that's where the details of this particular assay, I believe, are reported. 1:34PM

Q Sure. And if you can just tell the court reporter what pages you're looking at when you're drawing 1:34PM

this is an e-mail chain that starts on June 9th from -- I guess that's one of your technicians, is that right, Jack? 1:41PM

A Yes.

Q Okay. And it's an e-mail to you and then and he -- he sends you some data and then you, in turn, forward it to Jennifer in the middle e-mail. Do you see that? 1:41PM

A Yes.

Q Okay. And you write, "Hey Jen, I've sorted through the data and the replicates look weird and aren't tight at all." What do you mean when you characterize a replicate as looking weird? 1:41PM

A Well, without seeing the data, I don't know. I need the original Tad 2 file and I could tell you what I meant by weird. 1:42PM

Q Do you recall this e-mail?

A Yes. I do recall this series of events. And, again, it was all having to do with these composite water samples, I just can't remember what exactly the details of it was. 1:42PM

Q Okay. And would the same thing go for describing something as not being tight?

A Yes. So that is -- yes. So, like I said, if I had the Tad 2 file we could go through and I could tell 1:42PM

you exactly what I meant by that. 1:42PM
Q Okay. The next sentence you say, "In addition, the trends are messy and aren't very consistent." What kind of trends would you be looking for?
A Again, I don't know without seeing the data. 1:42PM
Q Okay. Do you need to see the data, the specific data to know what kind of trends you would be looking for?
A Yeah.
Q Okay. 1:42PM
A I mean, again, this is all in the biomarker development. We ran a lot of different experiments. So I do need some context and I do need to look at the data, specifically to provide a rationale for why I wrote what I did in this e-mail. 1:43PM
Q Okay. Let me direct you to the second to the last sentence of this e-mail. You say, "Bottom line -- it looks like the ATCC culture was amplifying better than your positive control and amplified to a higher end yield temperature." What does that mean? 1:43PM
A I don't know without looking at the data. Again, without knowing exactly what we were doing at this point in time, I can't say what this means. I need the backup. And we should be able to, if you have the lab notebooks, go back to these dates and we could figure 1:43PM

out what was going on at the time. 1:43PM

Q Okay. Let me get you to flip two pages more and you should have an e-mail that -- this page should be an e-mail starting from Travis Metal. Do you see that?

A Yes. 1:44PM

Q Okay. And this e-mail chain is exactly two pages long, as I understand it. This is the forwarding of a draft of the biomarker report. Do you agree with that characterization?

A Yes. 1:44PM

Q And then the next document you have should be the actual draft?

A Okay.

Q Flip to Page 22. Okay. And you see again that chart that we were talking about a few minutes ago? 1:45PM

A Yes.

Q And then over the next few pages, Page 23, 24, 25, there are a bunch of melt curves, a melt curve graph. Do you see that?

A Yes. 1:45PM

Q Okay. Now, these curves were not included in the final version, Exhibit 1A. You can check that if you want to.

A Uh-huh.

Q I will represent that they weren't. Do you recall 1:45PM

why they were not? 1:45PM
A Let's see, so the first one we just must have liked -- it's just showing that there's variability in the melt curve between our biomarker and the Brevibacteria and KCI. So we probably just -- I'm just 1:45PM looking back to see what figure we had. So we just decided to show that as two individual figures, probably for clarity. High water sample LAL15 is the qPCR biomarker. So that is whatever that high water sample is in the biomarker. And if that is a soil 1:46PM sample in the biomarker. I believe, since those samples were composites and not actually individual samples, we did not include them in the report because we didn't feel that they would be representative of the individual sample. 1:46PM
Q Okay. Go ahead and flip to the next e-mail. I think the report has 30 pages in it. And then after Pages 30 you'll find the next e-mail. Tell me when you get there.
A Okay. 1:47PM
Q This is e-mail dated September 27, 2007, and it's from Jennifer Weidhass to you. Do you see that?
A Yes.
Q And she's making a suggestion that it's to the draft report here. 1:47PM

A Okay." 1:47PM
Q And they're listed one through five. And then after number five, there's some text. Would you read that for me?
A "I think we need concurrence from the client regarding reporting the variability and melt curves in this report. This is something we have internal checks for and report for SOP in the qPCR reports. I think that this will be more ammunition to the defense and any expert they hired should know to ask to see melt 1:47PM curves rather than us suggesting it to them."
Q Why would inclusion of variability of melt curves in the report giving ammunition to the defense?
A I'm not exactly sure in the context, especially considering that we report melt curves as part of our 1:47PM standard reporting practice and chose to do that. I think at the time Jennifer was just thinking it was a bad thing if we were amplifying things that weren't specific to our marker, but in essence it is what it is and that's what we report when we see it, so -- 1:48PM
Q Do you recall this e-mail?
A Not specifically.
Q Okay. Again, what you've just told me is your speculation as to what she was thinking. Do you have any other recollections specific to this e-mail? 1:48PM

A A recollection in terms of? 1:48PM
Q Do you recall any other context about this e-mail?
A Context.
Q Do you remember -- sorry. Let me try this again.
Do you remember having a discussion with anyone 1:48PM
regarding whether variability of melt curves should be
included in your report?
A Not specifically, although clearly the decision
was made to report it as it was.
Q Okay. Do you recall discussing -- time to change 1:49PM
the tape then we'll continue.
THE VIDEOGRAPHER: We are going off the
record. The time is 1:49.
(Whereupon, a discussion was held off
the record.) 1:49PM
THE VIDEOGRAPHER: We are now on the
record. The time is 1:50 p.m.
Q Do you remember discussing -- other than this
e-mail, do you remember discussing with Jennifer
whether the issue of variability in melt curves should 1:49PM
be included in your report?
A In my mind, it was never a question to report it
or not. It is -- the melt curve analysis is an
essential component of the overall data assessment and
so it absolutely should be reported. 1:50PM

what you're asking? 3:05PM

Q No. You had the samples and you tested them using the qPCR assay?

A Uh-huh.

Q Then you came up with the report, the results which are charted on these results that you sent to your client that we've been looking at? 3:06PM

A Mm-hmm.

Q Did you perform any further analysis as to the meaning of the results at North Wind? 3:06PM

A Not that I'm aware of. We did collaborate some with Jody on the first paper so -- but in terms of, you know, further analysis, I guess I'm -- it just depends on what you mean.

Q Was anyone at North Wind responsible for concluding, based on this data, that -- that bacterial contamination from poultry litter is in location X, Y or Z? 3:06PM

A No, that was Jody Harwood's purview.

Q Do you recall Roger Olsen sharing with you analyses that Jody Harwood performed on your test results? 3:07PM

A Roger, I think, sent me an e-mail once showing the correlation between our marker and E. coli and maybe -- E. coli and maybe salmonella or something else. 3:07PM

Q Do you recall discussing that e-mail with anyone? 3:07PM
A Not specifically.
Q Do you recall having any reaction to it, agreeing, disagreeing?
A We probably, you know, looked at the data and saw 3:08PM the correlation and said, oh, that's interesting. You know, like I said, at this point we're not experts in microbial source tracking so our purview was really to implement the tools and to provide the data.
Q I've handed you Exhibit 14, which again is a 3:08PM collection of e-mails, and the first one is an e-mail from David Page to Roger Olsen copied to you and Christopher Teaf and Valerie Harwood and the e-mail just says, "Please see Jody's analysis." And then -- that's actually at the bottom e-mail of the page and 3:08PM the top e-mail is from David Page to you, again attaching the file called qPCR bacteria analysis. Do you see that?
A Mm-hmm.
Q And then what I provided you, the attachment to 3:09PM that is the Excel spreadsheet that was attached.
A Okay.
Q Do you recall this?
A Yeah.
Q If you flip to the middle of the Excel 3:09PM

spreadsheet, you'll actually see the correlating chart I think you were referring to. Do you see that? 3:09PM

A Yes.

Q Okay. Now, do you remember receiving this?

A Yes. 3:09PM

Q Okay. And this is what you were -- you were recollecting just a second ago?

A Yes.

Q Okay. Now, if you flip to -- let's see, the last two pages of this packet, you will see that it's the same e-mail chain. But that you have forwarded it to Jennifer on January 28, 2008, at 3:09 p.m.? 3:09PM

A Mm-hmm.

Q And then she writes back to you that same day at 5:19 p.m. Do you see that? 3:10PM

A Uh-huh.

Q Okay. Do you have any recollection of discussing this analysis from Dr. Harwood with Jennifer Weidhass?

A I don't.

Q Okay. Let me read the e-mail. She writes, "Actually, I don't think this is all that bad. When the biomarker is quantifiable the correlation with the coliforms is fairly good for environmental data. Also, there could be other sources of coliforms in the watershed that will contribute to the fecal material 3:10PM 3:10PM

count, but that are not the poultry litter. This is not good for the litigation against poultry farmers, i.e., other sources of fecal material, but doesn't have any bearing on the validity of the biomarker." 3:10PM

MR. BULLOCK: We're going to revoke your law license. 3:10PM

MR. TODD: What's that?

MR. BULLOCK: We're going to revoke her law license. She really doesn't give very good legal advice. 3:10PM

MR. TODD: I'm sure that she warmly and accurately reported her reaction to this.

MR. BULLOCK: Well, it may have been hers but I don't think any lawyer would have reacted like that. 3:11PM

MR. TODD: Let's get the witness's impression of this.

MR. BULLOCK: Oh, sorry.

Q Do you recall receiving this e-mail?

A Yes. 3:11PM

Q Okay. The first thing she wrote is, "Actually I don't think this is all that bad." Was she referring to a conversation that you had had previously with her regarding this data?

A I don't think so, no. 3:11PM

Q See right in the middle of this that there could be other sources of coliform in the watershed that would contribute to the fecal material count but that are not the poultry litter. What types of sources do you think she could be referring to? 3:11PM

MR. BULLOCK: Objection to form.

A I don't know. Honestly, I think this is just Jennifer's reaction to the data. We never really discussed it at length, her and I, so I can't really infer what she means specifically. 3:11PM

Q Okay. Do you agree with her that there can be sources of fecal coliforms in the watershed other than poultry litter?

MR. BULLOCK: Objection to form.

A I do not have an opinion. 3:12PM

Q You don't have an opinion whether poultry litter is the only source of fecal coliform bacteria in the Illinois River Watershed?

A No, I don't.

Q Are you familiar with what fecal coliforms are? 3:12PM

A Yes.

Q What are fecal coliforms?

A They are bacteria that are associated with different types of manure and waste, some of them have been associated with various pathogens or been 3:12PM

associated to be pathogens to humans. 3:12PM

Q Are they specific to chickens and turkeys?

MR. BULLOCK: Objection to form.

A No.

Q Do other types of animals carry them? 3:12PM

A It's my understanding, yes.

Q Is it possible that there are other types of animals in the Illinois River Watershed that would shed fecal coliforms?

A Yes. 3:13PM

Q So are poultry litter the only source of fecal coliforms in the Illinois River Watershed?

MR. BULLOCK: Objection to form.

A No.

MR. BULLOCK: Sorry. I'm sorry. I'm 3:13PM impinging on his space.

Q Based on the correlation chart that was forwarded to you in the prior e-mail here, what impact -- or, do you have an opinion as to what impact the fact that there are other sources of coliform, fecal coliform in 3:13PM the watershed would have on the suggestion that bacteria found in watershed derives from poultry litter?

A I do not.

Q Do you recall discussion at any point regarding -- 3:14PM

using PCR techniques to test environmental samples for the presence of salmonella or campylobacter? 3:14PM

A Yes.

Q Whose idea or who proposed the idea of doing that, if you know? 3:14PM

A We discussed it. I'm not sure who initially proposed the idea, but we did discuss it between Jennifer, Jody, myself and maybe others. I'm not sure who all were on the calls when we were discussing that.

Q Do you recall North Wind investigating whether this was possible? 3:14PM

A Yes.

Q And is it possible?

A We had identified some PCR methods. Jody had identified some PCR methods but we never tested any of those methods. 3:14PM

Q Okay. I've handed you, Dr. Macbeth, a packet of e-mails again from your production, all of which have to do with this testing. And I want to ask you a few questions about them. 3:15PM

A Yes.

Q The first e-mail is from Roger Olsen to you and Jennifer Weidhass dated March 14, 2008. And the second -- picking up with the second sentence, it reads, "In addition, Jody has talked with you about 3:15PM